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(54) Title: CYCLIC PEPTIDE ANALOGS OF SOMATOSTATIN

(57) Abstract

A cyclic peptide analog of somatostatin wherein a disulfide bond links the N-terminus residue and the C-terminus residue.

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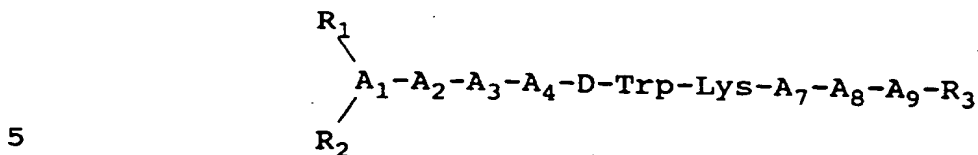## CYCLIC PEPTIDE ANALOGS OF SOMATOSTATIN

Background of the Invention

Native somatostatin is comprised of both a 14-
amino acid isoform (somatostatin-14) and a 18-amino acid
isoform (somatostatin-28). Heiman, et al.,
Neuroendocrinology, 45:429-436 (1987). Because of the
short half-life of the native somatostatin, various
somatostatin analogs have been developed, e.g., for the
treatment of acromegaly. Raynor, et al., Molecular
Pharmacol. 43:838 (1993). Five distinct somatostatin
receptors have been identified and characterized. Hoyer,
et al., Naunyn-Schmiedeberg's Arch. Pharmacol., 350:441
(1994). Somatostatin produces a variety of effects,
including modulation of hormone release, e.g., growth
hormone, glucagon, insulin, amylin, and neurotransmitter
release. Some of these effects have been associated with
its binding to a specific somatostatin receptor. For
example, the inhibition of growth hormone has been
attributed to the somatostatin type-2 receptor ("SSTR-2")
(Raynor, et al., Molecular Pharmacol. 43:838 (1993);
Lloyd, et al., Am. J. Physiol. 268:G102 (1995)) while the
inhibition of insulin has been attributed to the
somatostatin type-5 receptor ("SSTR-5") (Coy, et al.
197:366-371 (1993)). It is preferred to have an analog
which is selective for the specific somatostatin receptor
subtype responsible for the desired biological response,
thus, reducing interaction with other receptor subtypes
which could lead to undesirable side effects.

Summary of the Invention

The invention relates to a peptide covered by the
following generic formula:

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$$\begin{array}{c} R_1 \\ \backslash \\ A_1-A_2-A_3-A_4-D-Trp-Lys-A_7-A_8-A_9-R_3 \\ / \\ R_2 \end{array}$$

in which

A_1 is the D- or L-isomer of Cys, or Mpa;

A_2 is Asn, Gln, an aliphatic amino acid, an aromatic amino acid, or deleted;

A_3 is an aromatic amino acid;

A_4 is His or an aromatic amino acid;

A_7 is Thr, Ser, or an aliphatic amino acid;

A_8 is an aromatic amino acid;

A_9 is the D- or L-isomer of Cys;

each of R_1 and R_2 is, independently, H, C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} naphthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenylalkyl, C_{11-20} hydroxynaphthylalkyl, or COE_1 where E_1 is C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} naphthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenylalkyl, or C_{11-20} hydroxynaphthylalkyl; and

R_3 is NH_2 or $NH \cdot Y \cdot CH_2 \cdot Z$ where Y is a C_{1-12} hydrocarbon moiety (divalent, e.g., straight or branched alkyl group) and Z is H, OH, CO_2H, or $CONH_2$; and

a disulfide bond links the side chains of residues A_1 and A_9; or a pharmaceutically acceptable salt thereof.

In one embodiment, each of A_3 and A_8, independently, is Phe, p-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2), o-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2), pyridyl-Ala, Trp, β-Nal, 2,4-dichloro-Phe, Tyr(I), or F_5-Phe, A_4 is His, Phe, p-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2), o-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2), pyridyl-Ala, Trp, β-Nal, 2,4-dichloro-Phe, Tyr(I), or F_5-Phe, A_2 is Asn, Gln, Ala, Aib, Val, Leu, Ile, Nle, Nva, Abu, Phe, p-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2), o-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2), pyridyl-

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Ala, Trp, β-Nal, 2,4-dichloro-Phe, Tyr(I), F_5-Phe, or is
deleted, and A_7 is Thr, Ser, Ala, Aib, Val, Leu, Ile,
Nle, Nva, or Abu.

In a further embodiment, A_9 is Cys, each of A_3 and
5   A_8, independently, is Phe, p-X-Phe (where X is a halogen,
OH, or CH_3), Tyr(I), or Trp, A_4 is His, Phe, p-X-Phe
(where X is a halogen, OH, or CH_3), Tyr(I), or Trp, A_2 is
Asn, Gln, or is deleted, and A_7 is Thr or Ser.

In a still further embodiment, A_2 is Asn or is
10   deleted, A_3 is Phe, A_4 is Phe, His, p-X-Phe (where X is a
halogen, OH, or CH_3), Tyr(I), or Trp, A_8 is Phe or p-X-Phe
(where X is a halogen, OH, or CH_3), and each of R_1 and R_2,
independently, is H, and R_3 is NH_2.

Below are examples of the peptides of this
15   invention as covered by the above formula:

H_2-c[Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2
(Analog I),

H_2-c[D-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2
(Analog II),

20   H_2-c[Cys-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH_2
25   (Analog IV),

H_2-c[D-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH_2
(Analog III),

H_2-c[D-Cys-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH_2,
30   H_2-c[Cys-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-NH_2
(Analog VIII),

H_2-c[D-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-NH_2,
35   H_2-c[Cys-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

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H$_2$-c[Cys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$
(Analog V),

H$_2$-c[D-Cys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$,

H$_2$-c[Cys-Asn-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$,

5   H$_2$-c[D-Cys-Asn-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$,

H$_2$-c[Cys-Asn-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$,

H$_2$-c[D-Cys-Asn-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$,

H$_2$-c[Cys-Asn-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH$_2$,

H$_2$-c[D-Cys-Asn-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH$_2$,

10   H$_2$-c[Cys-Asn-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH$_2$,

H$_2$-c[D-Cys-Asn-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH$_2$,

H$_2$-c[Cys-Asn-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH$_2$,

H$_2$-c[D-Cys-Asn-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH$_2$,

H$_2$-c[Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-

15 NH$_2$,

H$_2$-c[D-Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-

NH$_2$,

H$_2$-c[Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-

NH$_2$,

20   H$_2$-c[D-Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-

NH$_2$,

H$_2$-c[Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$
(Analog VI),

H$_2$-c[Cys-Phe-β-Nal-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$
25 (Analog VII),

H$_2$-c[Cys-Phe-Cpa-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$
(Analog IX),

H$_2$-c[Mpa-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$
(Analog X),

30   H$_2$-c[Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-D-Cys]-NH$_2$
(Analog XI),

H$_2$-c[Cys-Phe-Phe-D-Trp-Lys-Thr-Tyr-Cys]-NH$_2$
(Analog XII),

H$_2$-c[Cys-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH$_2$
35 (Analog XIII),

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With the exception of the N-terminal amino acid, all abbreviations (e.g., Ala or A_2) of amino acids in this disclosure stand for the structure of -NH-CH(R)-CO-, wherein R is a side chain of an amino acid (e.g., CH_3 for
5 Ala). For the N-terminal amino acid, the abbreviation stands for =N-CH(CH_2SH)-CO- if it is the D- or L-isomer of Cys or

=C(-CH_2SH)-CO- if it is the D- or L-isomer of Mpa, wherein R is a side chain of an amino acid. Nle, Nva,
10 pyridyl-Ala, F_5-Phe, 2,4-dichloro-Phe, β-Nal, Abu, Mpa, Cpa, and Aib are respective abbreviations of the following α-amino acids: norleucine, norvaline, β-pyridyl-alanine, pentafluoro-phenylalanine, 2,4-dichlorophenylalanine, β-napthylalanine, α-aminobutyric
15 acid, mercaptopropionic acid, p-chlorophenylalanine, and α-aminoisobutyric acid. Tyr(I) refers to an iodinated tyrosine residue (e.g., 3-I-Tyr, 5-I-Tyr, 3,5-I-Tyr) wherein the iodine may be a radioactive isotope, e.g., I^{125}, I^{127}, or I^{131}. An aliphatic amino acid is an α-amino
20 acid having one or two side chains which, independently, are hydrocarbons, e.g., a straight or branched chain of 1-6 carbons. Examples of aliphatic amino acids include Ala, Aib, Val, Leu, Ile, Nle, Nva, or Abu. An aromatic amino acid is an α-amino acid the side chain of which has
25 a neutral (e.g., not acidic or basic) aromatic substituent, e.g., a substituted or unsubstituted phenyl, naphthyl, or aromatic heterocycle group (e.g., pyridyl or indolyl). Examples of aromatic amino acids include Phe, p-X-Phe (where X is a halogen (e.g., F, Cl, or I), OH,
30 OCH_3, CH_3, or NO_2), o-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2), pyridyl-Ala, Trp, β-Nal, 2,4-dichloro-Phe, Tyr(I), F_5-Phe. Where the amino acid residue is optically active, it is the L-isomer that is intended unless otherwise specified. Also, in the above generic
35 formula, hydroxyalkyl, hydroxyphenylalkyl, and hydroxynaphthylalkyl may contain 1-4 hydroxy substituents, and COE_1 stands for -C=O·E_1. Exampl s of

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$-C=O \cdot E_1$ include, but are not limited to, p-hydroxy-
phenylpropionyl (i.e., $-C=O \cdot CH_2-CH_2-C_6H_4-OH$) and
phenylpropionyl. In the formula set forth herein, the
disulfide bond between the thiol group on the side chain
5   of residue A_1 (e.g., Mpa, D-Mpa, Cys, or D-Cys) and the
thiol group on the side chain of residue A_9 (e.g., Mpa, D-
Mpa, Cys, or D-Cys) is not shown. A peptide of this
invention is also denoted herein by another format, e.g.,
$H_2-c[Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2$, with the two
10 disulfide bonded residues (i.e., Cys) placed between the
two brackets following "c".

The peptides of the invention can be used to
inhibit the release of insulin in a subject (a mammal
such as a human patient). Thus, the peptides are useful
15 in the treatment of physiological conditions in which the
inhibition of the release of insulin is of benefit, e.g.,
type II diabetes. Also, peptides of the invention having
a Tyr(I) residue can be used to image cells containing
somatostatin receptors (e.g., SSTR-5). Such peptides of
20 the invention can be used either *in vivo* to detect cells
having somatostatin receptors (e.g., cancer cells) or *in
vitro* as a radioligand in a somatostatin receptor binding
assay.

The peptides of this invention can be provided in
25 the form of pharmaceutically acceptable salts. Examples
of such salts include, but are not limited to, those
formed with organic acids (e.g., acetic, lactic, maleic,
citric, malic, ascorbic, succinic, benzoic,
methanesulfonic, toluenesulfonic, or pamoic acid),
30 inorganic acids (e.g., hydrochloric acid, sulfuric acid,
or phosphoric acid), polymeric acids (e.g., tannic acid,
carboxymethyl cellulose, polylactic, polyglycolic, or
copolymers of polylactic-glycolic acids).

A therapeutically effective amount of a peptide
35 of this invention and a pharmaceutically acceptable
carrier substance (e.g., magnesium carbonate, lactose, or
a phospholipid with which th therapeutic compound can

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form a micelle) together form a therapeutic composition
(e.g., a pill, tablet, capsule, or liquid) for
administration (e.g., orally, intravenously,
transdermally, pulmonarily, vaginally, subcutaneously,
5 nasally, iontophoretically, or by intratracheally) to a
subject in need of the peptide. The pill, tablet, or
capsule can be coated with a substance capable of
protecting the composition from the gastric acid or
intestinal enzymes in the subject's stomach for a period
10 of time sufficient to allow the composition to pass
undigested into the subject's small intestine. The
therapeutic composition can also be in the form of a
biodegradable or nonbiodegradable sustained release
formulation for subcutaneous or intramuscular
15 administration. See, e.g., U.S. Patents 3,773,919 and
4,767,628 and PCT Application No. WO 94/00148.
Continuous administration can also be obtained using an
implantable or external pump (e.g., INFUSAID™ pump) to
administer the therapeutic composition. The peptide can
20 be administered prior to bedtime of the patient.

       The dose of a peptide of the present invention
for treating the above-mentioned diseases or disorders
varies depending upon the manner of administration, the
age and the body weight of the subject, and the condition
25 of the subject to be treated, and ultimately will be
decided by the attending physician or veterinarian. Such
an amount of the peptide as determined by the attending
physician or veterinarian is referred to herein as a
"therapeutically effective amount."

30   Also contemplated within the scope of this
invention is a peptide covered by the above generic
formula for both use in treating diseases or disorders
associated with the need to inhibit insulin release,
e.g., type II diabetes, and use in detecting somatostatin
35 receptors, e.g., radioimaging.

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Other features and advantages of the present
invention will be apparent from the detailed description
and from the claims.

Detailed Description of the Invention

5   The synthesis and use of somatostatin analogs of
this invention are well within the ability of a person of
ordinary skill in the art. Unless defined otherwise, all
technical and scientific terms used herein have the same
meaning as commonly understood by one of ordinary skill
10 in the art to which this invention belongs. Also, all
publications, patent applications, patents, and other
references mentioned herein are incorporated by
reference.

        It is believed that one skilled in the art can,
15 based on the description herein, utilize the present
invention to its fullest extent. The following specific
embodiments are, therefore, to be construed as merely
illustrative, and not limitative of the remainder of the
disclosure in any way whatsoever.

Synthesis of Somatostatin Analogs

20

        The synthesis of short peptides is well examined
in the peptide art. See e.g., Stewart, et al., Solid
Phase Peptide Synthesis (Pierce Chemical Co., 2d ed.,
1984). The following is the synthesis of Analog I.
25 Other peptides of the invention can be prepared in an
analogous manner by a person of ordinary skill in the
art.

        Benzylhydrylamine-polystyrene resin (Advanced
ChemTech, Inc., Louisville, KY) (1.1 g, 0.5 mmole) in the
30 chloride ion form was placed in the reaction vessel of an
Advanced ChemTech ACT 200 peptide synthesizer programmed
to deliver the following reagents/solvents: (a) methylene
chloride; (b) 33% trifluoroacetic acid in methylene
chloride (2 times for 1 and 25 min each); (c) methylene

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chloride; (d) ethanol; (e) methylene chloride; and (f)
10% triethylamine in chloroform.

    The neutralized resin was stirred with Boc-S-4-
methylbenzyl-Cys and diisopropylcarbodiimide (1.5 mmole
5 each) in methylene chloride for 1 h and the resulting
amino acid resin was then cycled through steps (a) to (f)
in the above wash program. The following amino acids
(1.5 mmole) were then coupled successively by the same
procedure: Boc-Phe, Boc-O-benzyl-Thr, Boc-N-
10 benzyloxycarbonyl-Lys, Boc-D-Trp, Boc-Phe, Boc-Phe, and
Boc-S-methylbenzyl-Cys. After washing and drying, the
completed resin weighed 1.6 g.

    The resin (1.6 g, 0.5 mmole) was then mixed with
anisole (5 ml), dithiothreitol (100 mg), and anhydrous
15 hydrogen fluoride (35 ml) at 0°C and stirred for 45 min.
Excess hydrogen fluoride was evaporated rapidly under a
stream of dry nitrogen, and the free peptide precipitated
and washed with ether. The crude peptide was then
dissolved in 500 ml of 90% acetic acid to which was added
20 a concentrated solution of I_2/MeOH until a permanent
brown color was observed. Excess I_2 was removed by
addition of ascorbic acid, and the solution evaporated to
a small volume which was applied to a column (2.5 x 90
cm) of SEPHADEX™ G-25 which was eluted with 50% AcOH.
25 Fractions containing a major component by ultraviolet
(UV) absorption and thin layer chromatography were then
pooled, evaporated to a small volume, and applied to a
column (1.5 x 70 cm) of VYDAC™ octadecylsilane silica
(10-15 μm). This column was eluted with a linear
30 gradient from 80 percent A and 20 percent B to 40 percent
A and 60 percent B where A is 0.1% trifluoroacetic acid
(TFA) in water and B is 79.9% acetonitrile, 20% water,
and 0.1% TFA. Fractions were examined by thin layer
chromatography (tlc) and analytical high performance
35 liquid chromatography (hplc) and pooled to give maximum
purity. Repeated lyophilization of the solution from

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water gave 95 mg of the product as a white, fluffy
powder.

The product is found to be homogenous by hplc and
tlc. Amino acid analysis of an acid hydrolysate and
5 matrix-assisted laser desorption mass spectrometry (MALDI
MS) confirmed the composition of the cyclic octapeptide
(MW calculated, 1077; MW found, 1080).

The following is the synthesis of Analog V.
Benzylhydrylamine-polystyrene resin (Advanced ChemTech,
10 Inc.) (0.7 g, 0.25 mmole) in the chloride ion form was
placed in the reaction vessel of an Advanced ChemTech ACT
200 peptide synthesizer programmed to deliver the
following reagents/solvents: (a) methylene chloride; (b)
33% trifluoroacetic acid in methylene chloride (2 times
15 for 1 and 25 min each); (c) methylene chloride; (d)
ethanol; (e) methylene chloride; and (f) 10%
triethylamine in chloroform.

The neutralized resin was stirred with Boc-S-
methylbenzyl-Cys and diisopropylcarbodiimide (1.5 mmole
20 each) in methylene chloride for 1 h and the resulting
amino acid resin was then cycled through steps (a) to (g)
in the above wash program. The following amino acids
(1.5 mmole) were then coupled successively by the same
procedure: Boc-Phe, Boc-O-benzyl-Thr, Boc-N-
25 benzyloxycarbonyl-Lys, Boc-D-Trp, Boc-Phe, Boc-Phe, Boc-
Asn, and Boc-S-methylbenzyl-Cys. After washing and
drying, the completed resin weighed 1.2 g. The peptide
resin was subjected to HF cleavage and I_2 cyclization as
described above. Column purification, as described
30 above, yielded 21 mg of the cyclic nonapeptide which was
found to be homogeneous by hplc and tlc. Amino acid
analysis of an acid hydrolysate and MALDI MS confirmed
the composition of the cyclic nonapeptide (MW calculated,
1192; found, 1192).

35   The synthesis of iodinated somatostatin analogs
at the tyrosine residue (e.g., the chloramine-T method)
is well documented and are within the ability of a person

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of ordinary skill in the art. See, e.g., Cz rnick, et
al., J. Biol. Chem. 258:5525 (1993) and European Patent
No. 389,180 B1.

5   Somatostatin Receptor Binding Assay
     (1)   Human SSTR-2 Binding Assay:
            CHO-K1 (ovary, Chinese hamster) cells were
     obtained from the American Type Culture Collection
     (ATCC)(Rockville, MD) (ATCC No. CCL61) and were
10   transfected with the human SSTR-2 cDNA, described in
     Yamada, et al., Proc. Natl. Acad. Sci. USA, 89:251-255
     (1992) and also available from ATCC (ATCC No. 79046),
     using standard techniques known in the molecular
     biological art. See, e.g., Patel, et al., Biochem.
15   Biophys. Res. Commun. 198:605 (1994). Crude membranes
     were prepared by homogenizing the human SSTR-2
     transfected CHO-K1 cells in 20 ml of ice-cold 50 mM Tris-
     HCl (Buffer A) with a POLYTRON™ homogenizer (Brinkmann
     Instruments, Westbury, NY) at setting 6, for 15 sec.
20   Additional Buffer A was added to obtain a final volume of
     40 ml, and the homogenate was centrifuged in a SORVAL™
     SS-34 rotor (DuPont, Newtown, CT) at 39,000 g for 10 min
     at 0-4°C. The resulting supernatant was decanted and
     discarded. The pellet was rehomogenized in ice-cold
25   Buffer A, diluted, and centrifuged as before. The final
     pellet was resuspended in the 10 mM Tris HCl and held on
     ice for the receptor binding assay.
            Aliquots of the membrane preparation were
     incubated for 90 min at 25°C with 0.05 nM [^{125}I-Tyr]MK-678
30   (2000 Ci/mmol; c[N-methyl-Ala-Tyr(I^{125})-D-Trp-Lys-Val-
     Phe]) in 50 mM HEPES (pH 7.4) containing a test peptide
     at various concentrations (e.g., 10^{-11} to 10^{-6} M), 10
     mg/ml bovine serum albumin (fraction V, Sigma Chemical
     Co., St. Louis, MO), $MgCl_2$ (5 mM), Trasylol (200 KIU/ml),
35   bacitracin (0.02 mg/ml), and phenylmethyl-sulphonyl
     fluoride (0.02 mg/ml). The final assay volume was 0.3
     ml. The incubations were terminated by rapid filtration

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through GF/C filters (pre-soaked in 0.3% polyethylenimine for 30 min) using a filtration manifold (Brandel, Gaithersburg, MD). Each tube and filter was then washed three times with 5 ml aliquots of ice-cold Buffer A.
5 Specific binding was defined as the total $[^{125}I\text{-Tyr}]MK\text{-}678$ bound minus that bound in the presence of 200 nM somatostatin-14.

The following test peptides were assayed: somatostatin-14, somatostatin-28, Analog I, Analog II,
10 Analog III, Analog IV, and Analog V. The structure of Analogs I-V are shown above. The Ki values for the test peptides were calculated by using the following formula:

$K_i = IC_{50}/[1+(LC/LEC)]$ where IC_{50} is the concentration of the test peptide required to inhibit 50 percent of the
15 specific binding of the radioligand $[^{125}I\text{-Tyr}]MK\text{-}678$, LC is the concentration of the radioligand (0.05 nM), and LEC is the equilibrium dissociation constant of the radioligand (0.155 nM). The K_i values calculated for the test peptides are shown in the column under the heading
20 "SSTR-2" in
Table I.

(2)   <u>Human SSTR-5 Binding Assay</u>

CHO-K1 cells were transfected with the human SSTR-5 cDNA, described in Yamada, et al., Biochem
25 Biophys. Res. Commun., 195-844 (1993) using standard techniques known in the molecular biological art. See, e.g., Patel, et al., Biochem. Biophys. Res. Comm. 198:605 (1994). Crude membranes were prepared by homogenization of the human SSTR-5 transfected CHO-K1 cells in 20 ml of
30 ice-cold 50 mM Tris-HCl with a POLYTRON™ homogenizer (setting 6, 15 sec). Buffer was added to obtain a final volume of 40 ml, and the homogenate was centrifuged in a SORVAL™ SS-34 rotor at 39,000 g for 10 min at 0-4°C. The resulting supernatant was decanted and discarded. The
35 pellet was rehomogenized in ice-cold buffer, diluted, and

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centrifuged as before. The final pellet was resuspended in the 10 mM Tris HCl and held on ice for the receptor binding assay.

 Aliquots of the membrane preparation were
5 incubated for 30 min at 30°C with 0.05 nM [^{125}I-Tyr^{11}]somatostatin-14 (2000 Ci/mmol; Amersham Corp., Arlington Heights, IL) in 50 mM HEPES (pH 7.4) containing a test peptide at various concentrations (e.g., 10^{-11} to 10^{-6} M), 10 mg/ml bovine serum albumin (fraction V), MgCl$_2$
10 (5 mM), Trasylol (200 KIU/ml), bacitracin (0.02 mg/ml), and phenylmethyl-sulphonyl fluoride (0.02 mg/ml). The final assay volume was 0.3 ml. The incubations were terminated by rapid filtration through GF/C filters (pre-soaked in 0.3% polyethylenimine for 30 min) using a
15 Brandel filtration manifold. Each tube and filter was then washed three times with 5 ml aliquots of ice-cold buffer. Specific binding was defined as the total [^{125}I-Tyr^{11}]somatostatin-14 bound minus that bound in the presence of 1000 nM of the somatostatin type-5 receptor
20 ligand BIM-23052 (H$_2$-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH$_2$). The K$_i$ values for the test peptides were calculated by using the following formula: IC$_{50}$/[1+(LC/LEC)] where IC$_{50}$ is the concentration of the test peptide required to inhibit 50 percent of the specific binding of the
25 radioligand [^{125}I-Tyr^{11}]somatostatin-14, LC is the concentration of the radioligand (0.05 nM), and LEC is the equilibrium dissociation constant of the radioligand (0.16 nM). The K$_i$ values calculated for the test peptides are shown in the column under the heading "SSTR-
30 5" in Table I.

 Table I also shows the respective ratios of the K$_i$'s for the human SSTR-2 and the K$_i$'s for the human SSTR-5. The peptides of the invention (e.g., Analogs I-V) have ratios unexpectedly greater than one and, thus, are
35 more selective for the SSTR-5 than for the SSTR-2.

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TABLE 1

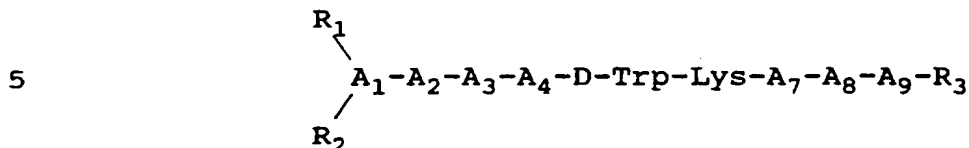| COMPOUND	SSTR-2	SSTR-5	SSTR-2/SSTR-5
Somatostatin-14	0.187	0.883	0.212
Somatostatin-28	0.242	0.383	0.632
Analog I	15.1	0.376	40.2
Analog II	13.0	2.63	4.94
Analog III	14.7	1.21	12.1
Analog IV	19.3	0.928	20.8
Analog V	129	2.43	53.1
Analog VI	6.19	0.34	18.2
Analog VII	8.07	4.145	1.94
Analog VIII	4.78	0.27	17.7
Analog IX	6.205	4.77	1.30
Analog X	15.0	0.744	20.1
Analog XI	34.83	28.04	1.24
Analog XII	59.20	5.78	10.2
Analog XIII	32.32	2.01	16.1

Other Embodiments

It is to be understood that while the invention
has been described in conjunction with the detailed
description thereof, that the foregoing description is
intended to illustrate and not limit the scope of the
invention, which is defined by the scope of the appended
claims. Other aspects, advantages, and modifications are
within the claims.

What is claimed is:

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CLAIMS

1. A p ptide of the formula:

$$\begin{array}{c} R_1 \\ \backslash \\ A_1-A_2-A_3-A_4-D-Trp-Lys-A_7-A_8-A_9-R_3 \\ / \\ R_2 \end{array}$$

in which

A_1 is the D- or L-isomer of Cys or Mpa;

A_2 is Asn, Gln, an aliphatic amino acid, an aromatic amino acid, or deleted;

A_3 is an aromatic amino acid;

A_4 is His or an aromatic amino acid;

A_7 is Thr, Ser, or an aliphatic amino acid;

A_8 is an aromatic amino acid;

A_9 is the D- or L-isomer of Cys;

each of R_1 and R_2 is, independently, H, C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} naphthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenylalkyl, C_{11-20} hydroxynaphthylalkyl, or COE_1 where E_1 is C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} naphthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenylalkyl, or C_{11-20} hydroxynaphthylalkyl; and

R_3 is NH_2 or $NH \cdot Y \cdot CH_2 \cdot Z$ where Y is a C_{1-12} hydrocarbon moiety and Z is H, OH, CO_2H, or $CONH_2$; and

a disulfide bond links the side chains of residues A_1 and A_9; or a pharmaceutically acceptable salt thereof.

2.   A peptide of claim 1, wherein each of A_3 and
A_8, indep ndently, is Ph , p-X-Phe (wh re X is a halogen,
OH, OCH_3, CH_3, or NO_2), o-X-Phe (where X is a halogen, OH,
OCH_3, CH_3, or NO_2), pyridyl-Ala, Trp, β-Nal, 2,4-dichloro-
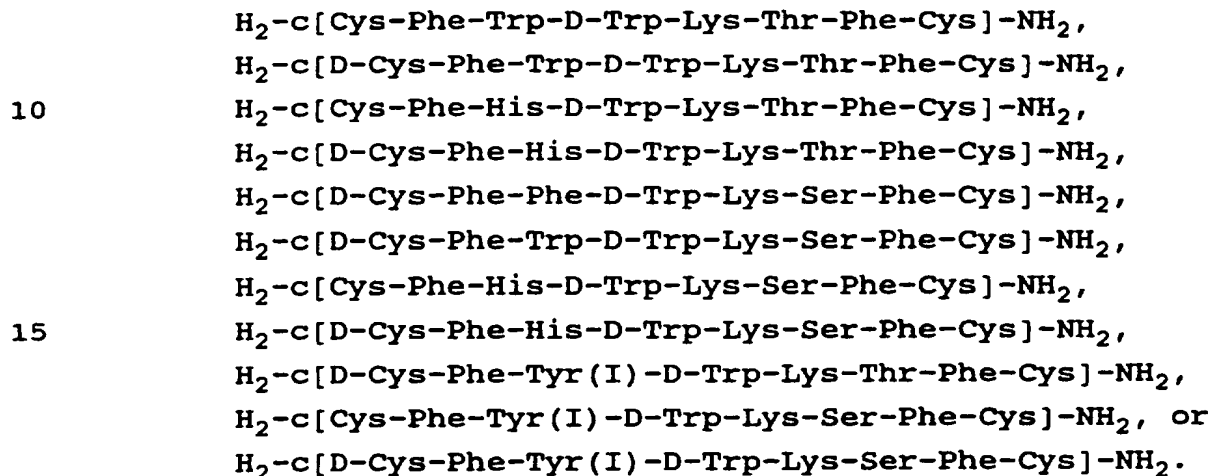5 Phe, Tyr(I), or F_5-Phe, and A_4 is His, Phe, p-X-Phe (where
X is a halogen, OH, OCH_3, CH_3, or NO_2), o-X-Phe (where X
is a halogen, OH, OCH_3, CH_3, or NO_2), pyridyl-Ala, Trp, β-
Nal, 2,4-dichloro-Phe, Tyr(I), or F_5-Phe.

3.   A peptide of claim 2, wherein A_2 is deleted,
10 and A_7 is Thr, Ser, Ala, Aib, Val, Leu, Ile, Nle, Nva, or
Abu.

4.   A peptide of claim 3, wherein A_9 is Cys.

5.   A peptide of claim 4, wherein each of A_3 and
A_8, independently, is Phe, p-X-Phe (where X is a halogen,
15 OH, or CH_3), Tyr(I), or Trp, and A_4 is His, Phe, p-X-Phe
(where X is a halogen, OH, or CH_3), Tyr(I), or Trp.

6.   A peptide of claim 5, wherein A_7 is Thr or
Ser.

7.   A peptide of claim 6, wherein A_3 is Phe, A_4 is
20 Phe, p-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or
NO_2), His, Tyr(I), or Trp, and A_8 is Phe or p-X-Phe (where
X is a halogen, OH, OCH_3, CH_3, or NO_2).

8.   A peptide of claim 6, wherein A_3 is Phe, A_4 is
Phe, Tyr, Tyr(I), or Trp, and A_8 is Phe.

25   9.   A peptide of claim 8, wherein each of R_1 and
R_2, independently, is H, and R_3 is NH_2.

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10.   A peptide of claim 9 of the formula:

H_2-c[Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH_2, or

H_2-c[Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-NH_2.
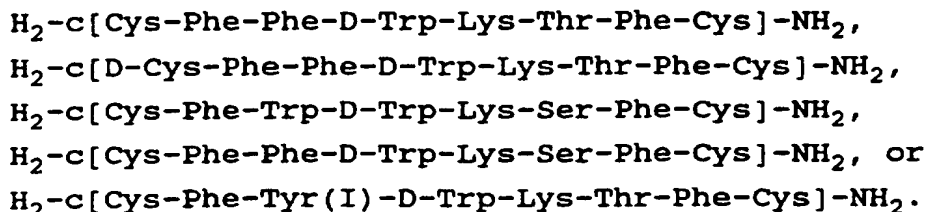
11.   A peptide of claim 7 of the formula:

H_2-c[Cys-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH_2,

H_2-c[D-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-NH_2,

H_2-c[Cys-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-NH_2, or

H_2-c[D-Cys-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-NH_2.

12.   A peptide of claim 2, wherein A_2 is Asn,
Gln, Ala, Aib, Val, Leu, Ile, Nle, Nva, Abu, Phe, p-X-Phe
(where X is a halogen, OH, OCH_3, CH_3, or NO_2), o-X-Phe
(where X is a halogen, OH, OCH_3, CH_3, or NO_2), pyridyl-
Ala, Trp, β-Nal, 2,4-dichloro-Phe, Tyr(I), or F_5-Phe, and
A_7 is Thr, Ser, Ala, Aib, Val, Leu, Ile, Nle, Nva, or
Abu.

13.   A peptide of claim 12, wherein A_9 is Cys.

14.   A peptide of claim 13, wherein each of A_3,
A_4, and A_8, independently, is Phe, p-X-Phe (where X is a
halogen, OH, or CH_3), Tyr(I), or Trp, and A_4 is His, Phe,
p-X-Phe (where X is a halogen, OH, or CH_3), Tyr(I) or
Trp.

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15.   A peptide of claim 14, wherein A_2 is Asn or Gln, and A_7 is Thr or S r.

16.   A peptide of claim 15, wherein A_3 is Phe, A_4 is Phe, p-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or 5 NO_2), His, Tyr(I), or Trp, and A_8 is Phe or p-X-Phe (where X is a halogen, OH, OCH_3, CH_3, or NO_2).

17.   A peptide of claim 16, wherein A_3 is Phe, A_4 is Phe, Tyr, Tyr(I), or Trp, and A_8 is Phe.

18.   A peptide of claim 17, wherein A_2 is Asn, 10 each of R_1 and R_2, independently, is H, and R_3 is NH_2.

19.   A peptide of claim 18 of the formula:
H_2-c[Cys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2.

20.   A peptide of claim 16 of the formula:
H_2-c[D-Cys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2,
15   H_2-c[Cys-Asn-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH_2,
H_2-c[D-Cys-Asn-Phe-Trp-D-Trp-Lys-Thr-Phe-Cys]-NH_2,
H_2-c[Cys-Asn-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH_2,
H_2-c[D-Cys-Asn-Phe-His-D-Trp-Lys-Thr-Phe-Cys]-NH_2,
H_2-c[Cys-Asn-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH_2,
20   H_2-c[D-Cys-Asn-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys]-NH_2,
H_2-c[Cys-Asn-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH_2,
H_2-c[D-Cys-Asn-Phe-Trp-D-Trp-Lys-Ser-Phe-Cys]-NH_2,
H_2-c[Cys-Asn-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH_2,
H_2-c[D-Cys-Asn-Phe-His-D-Trp-Lys-Ser-Phe-Cys]-NH_2,
25   H_2-c[Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-
NH_2,

H_2-c[D-Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys]-
NH_2,

H_2-c[Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-
30 NH_2, or

H_2-c[D-Cys-Asn-Phe-Tyr(I)-D-Trp-Lys-Ser-Phe-Cys]-
NH_2.

21. A peptide of claim 1 of the formula:

H_2-c[Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2.

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :C07K 7/00
US CL   :530/311

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   530/311, 328
930/160

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
somatostatin, cyclic, analog, nonapeptide, octapeptide, cys-phe-phe-d-trp-lys-thr-phe-cys

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,798,821 (HARTMANN) 17 January 1989, column 2, structure (IV); column 1, line 40 to column 3 line 10.	1-14, 21
Y	US, A, 5,358,934 (BOROVSKY et al.) 25 October 1994, column 2, line 64 to column 3 line 2.	1-14, 21
Y	EP, A, 0021585 (AMERICAN HOME PRODUCTS CORP) 07 January 1981, page 2, lines 6-9.	1-14, 21
Y	AU 84721/91 B (SANDOZ LTD) 21 November 1991, page 26, line 27.	1-14, 21
Y	BE 900089-A (SANDOZ S.A.) 07 January 1985, page 13, line 18.	1-14, 21

[X]   Further documents are listed in the continuation of Box C.   [ ]   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 OCTOBER 1996	**04 NOV 1996**

Name and mailing address of the ISA/US	Authorized officer
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INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2523125, 16 September 1983, pages 21 and 22, claims 6 and 7 respectively.	1,2,12,13
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Y		-----------
		1-14, 21

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